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Role of Signal-to-Cutoff Ratios in Hepatitis C Virus Antibody Detection

Marco Moretti,[a] Barbara Pieretti,[a] Annamaria Masucci,[a] Davide Sisti,[b] Marco Rocchi,[b] and Ernesto Delprete[a]

Laboratorio Patologia Clinica, Ospedale S. Croce, A.O.R.M.N., Fano, Italy,[a] and Dipartimento SUAN, Sezione di Biomatematica, Università degli Studi di Urbino Carlo Bo, Urbino, Italy[b]

We reviewed results from 12,800 samples tested for hepatitis C virus (HCV) antibody detection in our laboratory by screening (Ortho chemiluminescence immunoassay [CIA]) and supplemental tests (Chiron recombinant immunoblot assay [RIBA]). We found that a signal-to-cutoff (S/Co) ratio of 10.3 was, in our setting, the most efficient cutoff point to improve the diagnostic algorithm of HCV infection.

Hepatitis C virus (HCV) infection is a major public health concern (2, 12): about 3% of the world's population has been infected, and there are more than 170 million chronic carriers (17).

HCV infection can lead to end-stage liver disease, cirrhosis, and hepatocellular carcinoma, and mortality will continue to increase over the next 2 decades (1, 10).

The diagnosis of HCV infection is based on serological assays that detect specific antibodies to HCV (anti-HCV) and molecular assays that detect viral nucleic acid (HCV RNA) (14). Testing for the presence of anti-HCV antibodies is recommended for initial identification of persons with HCV infection (3, 7, 16). Anti-HCV detection by immunoassay screening tests is generally the first step in clinical diagnosis and screening of asymptomatic subjects. Screening tests have high false-positive rates, particularly among populations with a low (<10%) prevalence of HCV infection (4). For this reason, more specific supplemental tests such as recombinant immunoblot assay (RIBA) or a nucleic acid test (NAT) using reverse transcriptase PCR (RT-PCR) for HCV RNA detection are used to confirm positive anti-HCV screening tests (15).

As many as nine testing strategies for detection of HCV infection have been analyzed (6).

The Centers for Disease Control and Prevention (CDC) published guidelines in order to provide a systematic approach for the laboratory diagnosis of HCV infection, suggesting algorithms for accurate, efficient, and cost-effective strategies using screening and supplemental tests (4).

Screening for anti-HCV antibodies is carried out in our laboratory using the Ortho Vitros anti-HCV 3.0 chemiluminescence assay (Ortho-Clinical Diagnostics, Johnson & Johnson, United Kingdom) on the Vitros ECiQ automated analyzer (Ortho chemiluminescence immunoassay [CIA]) (8, 11, 13). This is a two-step sandwich enhanced chemiluminescence immunoassay for the detection of human antibodies to several HCV recombinant antigens (c22-3, c200, and NS-5).

Results are calculated as normalized signal-to-cutoff (S/Co) ratios obtained by measuring the signal strength of sample and the signal strength of an internal cutoff. Samples with an S/Co ratio of ≥1.0 are defined by the manufacturer as positive.

Each positive sample by Ortho CIA screen is followed by Chiron RIBA HCV 3.0 strip immunoassay (Chiron Corporation, Emeryville, CA), a more specific supplemental anti-HCV assay to confirm screening test results.

TABLE 1 RIBA results in relation to the CIA S/Co ratio subset

CIA S/Co ratio (no. of samples)	No. (%) of RIBA results:		
	Negative	Indeterminate	Positive
≤3.0 (43)	31 (72.1)	11 (25.6)	1 (2.3)
3.01–8.0 (26)	8 (30.8)	16 (61.5)	2 (7.7)
8.01–20.0 (36)	4 (11.1)	8 (22.2)	24 (66.7)
>20.01 (208)	2 (1.0)	11 (5.3)	195 (93.7)
Total (313)	45	46	222

Chiron RIBA is a qualitative enzyme immunoblot assay for the detection of antibodies against recombinant antigens (c33c and NS5) and HCV-encoded synthetic peptides (c22, c100, and 5-1-1). The anti-HCV reactivity of specimens is determined by visually comparing each HCV band to the intensity of the low- and high-human-IgG internal control bands blotted onto each strip. A negative, indeterminate, or positive interpretation is based on the reaction pattern present on the strip.

The CDC guidelines (4) for laboratory testing reported that screening test positive results are classified as having high S/Co ratios if their ratios are at or above a predetermined value that predicts a supplemental test positive result ≥95% of the time, regardless of the anti-HCV prevalence or characteristics of the population being tested.

The CDC on its website (5) gives S/Co ratios predictive of a true positive ≥95% of the time for each screening test available. For Ortho CIA, high S/Co ratios are defined as ratios of ≥8.0.

Several studies have been published about the ability of this screening test to predict the supplemental test result (9, 11, 14, 15). Lai et al. (14) concluded that for Ortho CIA, it is not necessary to confirm negative or positive values if the S/Co ratio is <3.0 or ≥20.0 because of the high rate of true-negative and true-positive

Received 23 March 2012 Returned for modification 19 April 2012
Accepted 1 June 2012
Published ahead of print 13 June 2012
Address correspondence to Marco Moretti, marcomoretti18@libero.it.

doi:10.1128/CVI.00175-12

TABLE 2 Diagnostic performance of the CIA screening test in the prediction of RIBA results[a]

Parameter	% correct diagnosis (95% CI) at CIA S/Co ratio of:		
	3.0	8.0	20.0
Sensitivity	99.5 (97.5–100)	98.6 (96.1–99.7)	87.8 (82.8–91.8)
Specificity	46.2 (35.6–56.9)	72.5 (62.2–81.4)	85.7 (76.8–92.2)
PPV	81.9 (76.7–86.3)	89.8 (85.2–93.3)	93.8 (89.5–96.6)
NPV	97.7 (87.7–99.9)	95.7 (87.8–99.1)	74.3 (64.8–82.3)

[a] 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.

results, respectively; other authors suggested that confirmatory tests are not necessary for patients with S/Co ratios of <5.0 and <4.5 (15, 9).

The objective of the present study was to evaluate in our setting the relationship between Ortho CIA-positive S/Co samples and Chiron RIBA results to assess if our diagnostic algorithm might be modified in order to reduce unnecessary supplementary tests.

We retrospectively reviewed results from a database of 12,800 serum samples that were tested from 1 July 2008 to 31 December 2010. Of these, 7,000 samples (54.7%) were from hospitalized patients and 5,800 (45.3%) were from outpatients.

All samples were analyzed for anti-HCV antibodies screening detection using the Ortho CIA, and all positive sera were evaluated with the Chiron RIBA as a supplemental test.

Statistical analysis was carried out with Stata Release statistical software version 11.0 (Stata Corp. LP, College Station, TX) and Visual Basic (VBA) for Windows. A P value of <0.05 was considered significantly different.

Among 12,800 patients tested, 313 (2.4%) resulted positive (S/Co ratio, ≥1.0) by Ortho CIA. The S/Co ratio of positive samples ranged from 1.0 (minimum) to 30.1 (maximum). The mean value was 19.1 (standard deviation [SD], 9.4), and the 5th and 95th percentiles were 1.28 and 28.50, respectively.

Of the 313 Ortho CIA-positive patients, 222 (71.0%), 46 (14.7%), and 45 patients (14.3%) were positive, negative, and indeterminate, respectively, by Chiron RIBA.

We categorized positive samples on the basis of the S/Co ratio and calculated ratios of negative, indeterminate, and positive results by Chiron RIBA (Table 1).

The diagnostic sensitivity and specificity, the positive predictive value (PPV) and negative predictive value (NPV), were calculated at S/Co ratios of 3.0, 8.0, and 20.0, respectively (Table 2). Ordinal regression analysis was performed using the S/Co screening test as the continuous predictive variable and the confirmatory test as the ordinal dependent variable (0, negative; 1, indeterminate; 2, positive). The analysis with ordinal regression shows that the screening test value is strongly associated with the ordinal result of the confirmatory test ($\chi^2 = 226.1$, $P < 0.0001$), suggesting a strong relationship between screening and supplemental tests. Despite the relationship, there are a statistically significant number of samples with an indeterminate result (Fig. 1).

The values of the S/Co ratios associated with 95% PPV and 95% NPV were 10.3 and 3.0, respectively.

On the basis of our present study and literature data, we modified our algorithm for HCV testing.

If the S/Co ratio is <10.3, the Ortho CIA results should be confirmed by supplemental Chiron RIBA.

We decided to report specimens with an S/Co ratio of ≥10.3 without a supplemental test but with an explanatory comment. We declare in the comment that supplemental serological testing was not performed for a sample with an S/Co ratio of ≥10.3 since in these cases, the screening test predicts a true antibody-positive result ≥95% of the time. We inform the test-ordering physician also that more specific testing can be requested if necessary, especially for people being tested for HCV infection for the first time or on the basis of other clinical or laboratory information. For this eventuality, we store the specimens for the supplemental test. Therefore, we suggest performing reverse transcriptase-PCR (RT-

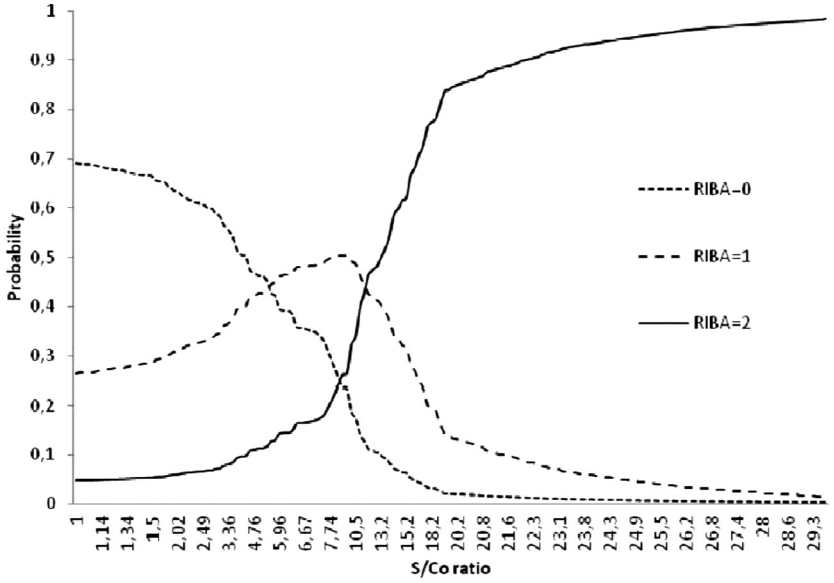

FIG 1 Plot of ordinal regression results: relationship between test screening (S/Co ratio) and RIBA results for HCV. The abscissa represents crescent values for screening test (minimum, 1.0; maximum, 30.1), while the horizontal line represents the results of RIBA. RIBA results are symbolized as follows: 0, negative; 1, indeterminate; and 2, positive.

PCR) to detect HCV viremia in positive samples at the screening test and with indeterminate or positive RIBA results.

We believe that implementation of this algorithm will improve the accuracy, efficiency, and utility of anti-HCV testing, providing more reliable results for physicians and their patients, and can reduce unnecessary supplementary testing. We also suggest that this type of validation will need to be done by each laboratory since the population characteristics and the assay used will both have an effect on the cutoff selection.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Elena Arighi and Vijay Kumar.

REFERENCES

1. **Brown RS.** 2005. Hepatitis C and liver transplantation. Nature **436**:973–978.
2. **Centers for Disease Control and Prevention.** 1991. Public Health Service interagency guidelines for screening donors of blood, plasma, organs, tissues, and semen for evidence of hepatitis B and hepatitis C. MMWR Morb. Mortal. Wkly. Rep. **40**:1–17.
3. **Centers for Disease Control and Prevention.** 1998. Recommendations for prevention and control of hepatitis C virus infection and HCV-related chronic disease. MMWR Morb. Mortal. Wkly. Rep. **47**:1–53.
4. **Centers for Disease Control and Prevention.** 2003. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus (anti-HCV). MMWR Morb. Mortal. Wkly. Rep. **52**:1–13.
5. **Centers for Disease Control and Prevention.** 2011. Hepatitis C information for health professionals: laboratory testing. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/hepatitis/HCV/LabTesting.html. Accessed December 2011.
6. **Chapko MK, et al.** 2005. Cost effectiveness of testing strategies for chronic hepatitis C. Am. J. Gastroenterol. **100**:607–615.
7. **Chien D, et al.** 1992. Diagnosis of hepatitis C virus (HCV) infection using an immunodominant chimeric polyprotein to capture circulating antibodies: reevaluation of the role of HCV in liver disease. Proc. Natl. Acad. Sci. USA **89**:10011–10015.
8. **Colin C, et al.** 2001. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assay: an analysis of the literature. J. Viral Hepat. **8**:87–95.
9. **Contreras AM, et al.** 2008. Very low hepatitis C antibody levels predict false-positive results and avoid supplemental testing. Transfusion **48**:2540–2548.
10. **Deuffic-Burban S, Poyinard T, Sulkowski MS, Wong JB.** 2007. Estimating the future health burden of chronic hepatitis C and human immunodeficiency virus infection in the United States. J. Viral Hepat. **14**:107–115.
11. **Dufour DR, Talastas M, Fernandez MDA, Harris B.** 2003. Chemiluminescence assay improves specificity of hepatitis C antibody detection. Clin. Chem. **49**:940–944.
12. **Ghany MG, Strader DB, Thomas DL, Seff LB.** 2009. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology **49**:1335–1374.
13. **Griffith BP, et al.** 2003. Hepatitis C virus antibody signal to cut-off ratios predictive of HCV RNA detection: comparison of the Ortho Vitros and EIA assays, abstr M48. Abstr. 19th Annu. Clin. Virol. Symp.
14. **Lai KKY, et al.** 2011. Improved reflexive testing algorithm for hepatitis C infection using signal-to-cutoff ratios of a hepatitis C virus antibody assay. Clin. Chem. **57**:1050–1056.
15. **Oethinger M, Mayo DR, Falcone J, Barua PK, Griffith BP.** 2005. Efficiency of the Ortho VITROS assay for detection of hepatitis C virus-specific antibodies increased by elimination of supplemental testing of samples with very low sample-to-cutoff ratios. J. Clin. Microbiol. **43**:2477–2480.
16. **Van der Poel CL, et al.** 1991. Confirmation of hepatitis C virus infection by new four antigen recombinant immunoblot assays. Lancet **337**:317–319.
17. **World Health Organization.** 2002. Gobal alert and response: hepatitis C. World Health Organization, Geneva, Switzerland. www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index4.html. Accessed December 2011.